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Our Ref.: C 1976 PCT

CLAIMS

- 1. A composition comprising
 - (a) a nucleic acid molecule encoding a fusion protein comprising
 - (aa) a (poly)peptide that enhances solubility and/or prevents aggregation of said fusion protein; and
 - (ab) an amyloidogenic (poly)peptide that has the ability to self-assemble into amyloid-like fibrils or protein aggregates; wherein the connection of both (poly)peptides (aa) and (ab) is via a linker or by a direct attachment, wherein further either the linker or either (poly)peptide comprises a cleavable site, or wherein said fusion protein comprises a number of cleavage sites and wherein said cleavable site(s) render both (poly)peptides essentially intact;
 - (b) a vector containing the nucleic acid molecule of (a);
 - (c) a host transformed with the vector of (b);
 - (d) a fusion protein encoded by the nucleic acid of (a) or a functional derivative thereof; and/or
 - (e) an antibody specific for the fusion protein of (d).
- 2. The composition of claim 1 wherein the amyloidogenic (poly)peptide comprises a polyglutamine expansion.
- 3. The composition of claim 2 wherein said polyglutamine expansion comprises at least 35 glutamines.
- 4. The composition of claim 3 wherein said polyglutamine expansion comprises at least 51 glutamines.
- 5. The composition of any one of claims 2 to 4 wherein said (poly)peptide defined in (ab) is huntingtin, androgen receptor, atropin, TATA binding protein, or ataxin-1,-2,-3, -6 or -7 or a fragment or derivative thereof.

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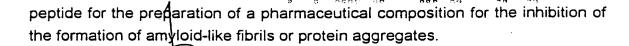
The composition of any one of claims 1 to 5 wherein said amyloidogenic (poly)peptide self-assembles subsequent to release from said fusion protein.

- 7. The composition of claim 1 wherein said amyloidogenic (poly)peptide is the amyloid precursor protein (APP), β-protein, an immunoglobulin light chain, serum amyloid A, transthyretin, cystatin C, β2-microglobulin, apolipoprotein A-1, gelsoline, islet amyloid polypeptide (IAPP), calcitonin, a prion, atrial natriuretic factor (ANF), lysozyme, insulin, fibrinogen, tau proteins or α-synuclein or a fragment or derivative thereof.
- 8. The composition of any one of claims 1 to 7 wherein said (poly)peptide defined in (aa) is glutathione S-transferase (GST), intein, thioredoxin, dihydrofolate reductase (DHFR) or chymotrypsin inhibitor 2 (CI2) or a functional fragment or derivative thereof.
- 9. The composition of any one of claims 1 to 8 wherein said nucleic acid is DNA.
- 10. The composition of any one of claim 1 to 9 wherein said vector is an expression vector or a gene targeting vector.
- 11. The composition of any one of claims 1 to 10 wherein said host is a bacterial, preferably an E.coli, an animal-, preferably a mammalian, an insect-, a plant-, a fungal, preferably a yeast and most preferably a Saccharomyces or Aspergillus cell, a Pichia pastoris cell, a transgenic animal or a transgenic plant.
- 12. A method of producing a fusion protein as defined in the composition of any of the preceding claims comprising culturing or raising the host as defined in claim 11 and isolating said fusion protein.
- 13. The composition of any one of claims 1 to 12 wherein said antibody is a monoclonal antibody, polyclonal antibody, phage display antibody or a fragment or derivative thereof.
- 14. An in vitro method of producing amyloid aggregates comprising
 - (a) at least partially cleaving the fusion protein comprised in the composition of any one of claims to 13 wherein the (poly)peptide that is released has the ability to self-assemble into amyloid-like fibrils or protein aggregates; or
 - (b) inducing self-assembly into amyloid-like fibrils or protein aggregates by heating the fusion protein comprised in the composition of any one of claims 1 to 13 or an amyloidogenic (poly)peptide that has the ability to self-assemble into amyloid-like fibrils or protein aggregates, by inducing a pH

change in a solution comprising said fusion protein/(poly)peptide or by treating said fusion protein/(poly)peptide with a denaturing agent.

- 15. The method of claim 14 wherein said cleavage is effected chemically or enzymatically, or by the intein self-cleavage reaction in the presence of thiols.
- 16. A method of testing a prospective inhibitor of aggregate formation of a fusion protein as defined in the composition of any one of claims 1 to 13 when enzymatically or chemically cleaved or a non-cleaved fusion amyloidogenic (poly)peptide as defined in any one of claims 1 to 13 comprising
 - (a) incubating in the presence of a prospective inhibitor of aggregate formation said fusion protein in the presence or absence of a cleaving agent; and
 - (b) assessing the formation of amyloid-like fibrils or protein aggregates.
- 17. The method of claim 16 wherein incubation is effected in the presence of factor Xa, trypsin, endoproteinase Arg-C, endoproteinase Lys-C, proteinase K or elastase at a temperature of preferably 25 to 37°C for 0,5 to 16 hours and the assessment of the formation of fibrils or aggregates in step (b) is preferably effected by a filter assay or by a thioflavine T (ThT) fluorescence assay, in which the fluorescence intensity reflects the degree of aggregation
- 18. A method for identifying an inhibitor of aggregate formation of a fusion protein as defined in any one of claims 2 to 6 prior to or after proteolytic or chemical cleavage or of a non-fusion amyloidogenic (poly)peptide that has the ability to self-assemble into amyloid-like fibrils or protein aggregates comprising
 - (a) loading a surface δ_{ζ} gel with said protein or an aggregate thereof;
 - (b) incubating said surface or gel with a prospective inhibitor; and
 - (c) assessing whether the presence of said prospective inhibitor avoids or reduces aggregate formation or further aggregate formation.
- 19. The method of claim 18 wherein said surface is a membrane.
- 20. Use of an antibody, pyrontine Y, guanidine hydrochloride, urea, rifampicin or a derivative thereof, myristyltrimethylammonium bromide, hydroquinone, p-benzoquinone, 1,4-dihydroxynaphthalene, p-methoxyphenol, α-tocopherol, ascorbic acid, β-carotene, anthracycline, doxorubicin, hexadecyl-N-methylpiperidinium, dodecyltrimethyl-ammonium, N-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, a (poly)peptide, glutamine or an oligoglutamine

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21. A transgenic mamma or plant comprising a nucleic acid molecule or vector as described in the composition of claim 3 or 4.